BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

FEBRUARY 1939

THE SIGNIFICANCE OF THE ALBUMIN FRACTION OF SERUM

Harvey Lecture, November 17, 1938

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SERUM ALBUMIN is to be defined as that fraction of the protein of serum which remains in solution after half saturation with ammonium sulphate or which is not salted out in a 21.5 per cent solution of sodium sulphate. The fractions which are removed by these procedures are globulins. Serum albumin, so defined, possesses physiologic characteristics which are distinct from those of the globulins. It is important in maintaining the volume of the blood and it is essential to the flow of fluid across the capillary wall. These properties are intimately concerned with the general phenomenon of the circulation of the blood, a circumstance which makes it peculiarly appropriate that they be discussed before the Harvey Society. Moreover, the physiologic properties of serum albumin are of great importance in medicine for the amount of albumin which circulates in the plasma is easily depleted when health is disordered. An understanding of this process of depletion, and its converse, replenishment, is essential to correct appraisal of the significance of the albumin fraction of serum.

In selecting material in a large field from which to prepare this eve-

ning's lecture, I have perforce given preference to investigations which have occupied the attention of workers in the laboratory which I represent. A few aspects only can be included of the important role of serum albumin in regulating the distribution of fluid between capillaries and tissue spaces; this phase of the subject has been covered in a recent Harvey Lecture by Eugene M. Landis.[1] Likewise, Cecil K. Drinker[2] has brought before you some of the problems which require elucidation in connection with the occurrence of serum proteins in lymph and I shall not enter this field of controversy. Since familiarity with ground to be covered will aid in orientation, I shall begin by presenting an outline:

Part I. Effect of diet on serum albumin concentration
- a. The process of depletion
- b. The process of replenishment

Part II. Physiologic importance of serum albumin
- a. Fluid distribution and edema
- b. Absolute and relative permeability of capillaries
- c. Regulation of blood volume

Part III. Medical control of serum albumin deficiency
- a. Injection of acacia; transfusions with serum and blood
- b. Diet; qualitative differences among food proteins in promoting albumin synthesis

Part I.

In 1929 Frisch, Mendel, and Peters[3] reported that young rats fed on diets composed chiefly of carrots developed deficits in serum protein. The diet was deficient in protein, but otherwise adequate. In 1931 Shelburne and Egloff[4] described the development of hypoproteinemia in a dog during maintenance on a low protein diet. These observations have been used in the laboratory of the Babies Hospital to provide a method[5, 6] for depleting the serum albumin. Our story can be opened by observing the method.

Dogs are maintained on a low protein diet, the composition of which is indicated in Table I. The quantities listed are the amounts given to each dog per day. Animals subsisting on the diet have always exhibited a negative balance of nitrogen. Metabolism observations indicate an average daily loss of nitrogen of 1.15 gm. The biological effects of subsistence on the diet appear to be referable to deficiency of protein alone. When the diet is supplemented with casein it has proved adequate for maintenance

of health. The addition of extra amounts of various vitamins[7] has not altered the course of experiments.

TABLE I

COMPOSITION OF LOW PROTEIN DIET

	grams
Carrots	300
Rice	35
Lard	40
Cod liver oil	10
Sugar	115
Salt mixture	5
Water, sufficient to make	900

Furnishes 1200 calories; 1.23 gm. nitrogen

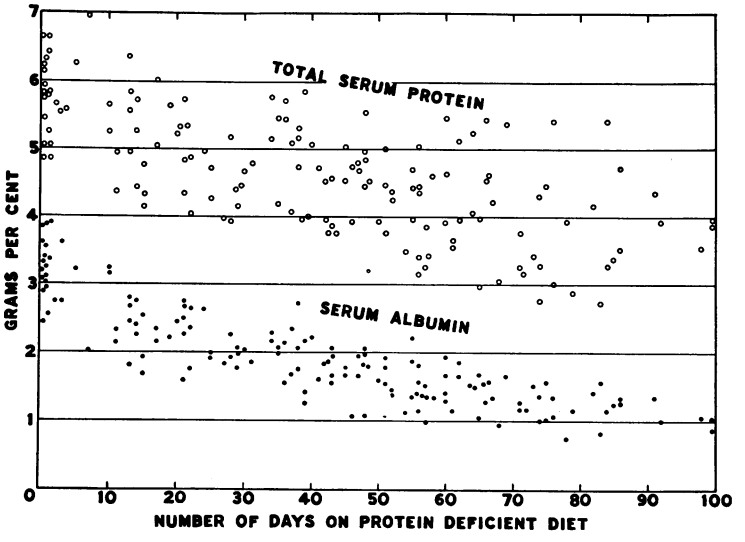

Chart 1—Concentration of albumin and total protein in the serum during maintenance on the low protein diet. From the *Journal of Experimental Medicine.*[6]

Chart 1 shows the results of 150 determinations of the albumin and total protein of serum in twenty-one dogs during maintenance on the diet. The downward trend of albumin and total protein with continuance of the diet is pictured clearly. In Chart 2 the trends are shown by average lines and the course of the globulin fraction is depicted. The average concentration of globulin remains singularly constant; it follows that the fall in total protein is brought about entirely by depletion of the albumin fraction.

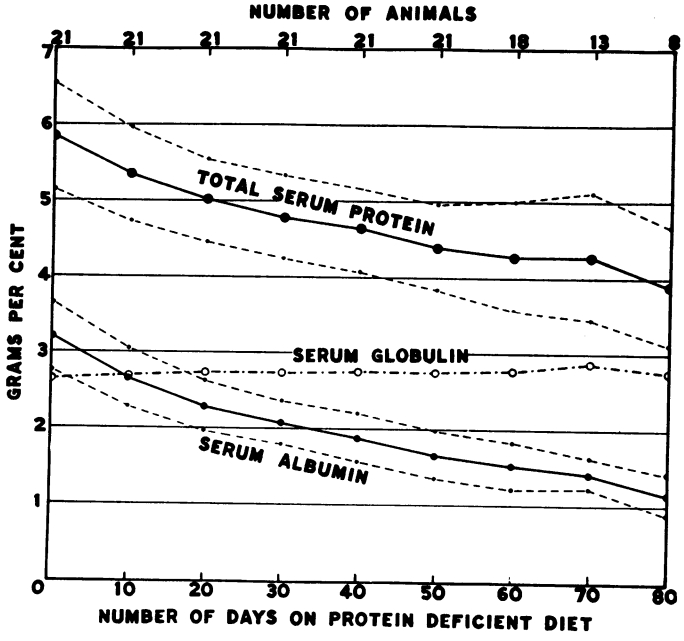

Chart 2—The average trends of albumin, globulin, and total protein during maintenance on the low protein diet. The dotted lines above and below the unbroken lines for albumin and total protein are placed at a distance of one standard deviation from the average values. From the *Journal of Experimental Medicine.*[6]

There are two additional points concerning the depletion of albumin. The first can be seen from the chart, namely, that the rate of decline is more rapid during the initial days or few weeks of maintenance on the diet than subsequently. On the average it requires eleven weeks to double the fall in concentration which develops in three weeks. The slowing in the rate of decline with the passage of time has been shown in metabolism studies to be associated with a progressive diminution in the nitrogen lost by the body. There is thus portrayed an adaptive ability of the body in adjusting its metabolic processes so as to spare protein. A similar adaptive ability has been described in humans[8] during subsistence on low protein diets and the process has been shown to be associated with a fall in the basal output of energy.[9, 10]

A second and curious fact concerning the depletion of albumin is that

the rate of fall is independent or almost independent of the amount of energy consumed in the diet. One might well have expected that a high intake of calories in the form of carbohydrate and fat would not only protect the body nitrogen but also slow to a minimum the depletion of protein in the serum. This does not appear to be the case. We have not been able to discern that the decline is more rapid during fasting than when the energy intake is liberal. Among eighty-three animals in which the daily dietary calories varied from fifty to eighty-seven per kilo of weight, the coefficient of correlation between energy intake and percentage albumin depletion after three weeks was only 0.11, a degree of association entirely devoid of statistical significance.

We have now seen that the simple procedure of maintaining a dog on a low protein diet not only allows the production of serum albumin deficits in a laboratory animal and thereby affords the means for observation of the consequences but also that some features which characterize the process of depletion can be made out. It will be instructive to watch the process in reverse so as to learn something of the nature of regeneration.

In the first group of experiments[11] moderate albumin depletion was obtained by administering the low protein diet to four dogs for a period of three weeks; the diet was then supplemented by the addition of beef so that each animal would receive eighty calories and 5 gm. protein per kilo. The degree of albumin depletion can be seen in Table II by comparing the initial levels in the first column with the levels in the second column which represent analyses at the end of the depletion period. Subsequent daily levels when the diet was supplemented with beef are shown in the remaining columns. The result was the same in each of the four experiments; it can be visualized best by a graph, Chart 3, showing the average course of regeneration. The course can be described with close approximation by a straight line. In two of the four experiments the regeneration was followed until the albumin had attained its previous level of health. Within the limits of experimental error there was no break in the straight line path of albumin concentration until this level was reached. Thereafter, no further regeneration occurred. These experiments are informative because they dispose of an idea which has been expressed or implied in recent literature, namely, that the strength of the stimulus for regeneration of albumin is proportional to the degree of albumin depletion. Apparently the stimulus is constant so long as any de-

TABLE II

Dog	Before Depletion	Days on Regeneration Diet 0	1	2	3	4	5	6	7	8	9	10	11	12
7-31	3.65	2.82	3.17	2.97	2.99	3.28	3.28	3.65	3.52	3.64	3.63		3.61	3.64
5-31	4.22	3.08	3.04	3.13	3.42	3.50	3.68	3.93	4.10	4.12	4.18	4.11	4.30	4.24
8-04	3.57	2.56	2.58	2.71	2.79	2.81	2.83	2.94	2.88	3.12	3.18	3.14		3.26
9-51	3.98	2.96	3.06	3.09	3.26	3.32	3.31	3.58	3.53	3.60	3.75			
Average	3.86	2.86	2.96	2.98	3.12	3.23	3.28	3.53	3.51	3.62	3.69			

Results of daily determinations of serum albumin concentration during regeneration on a diet which furnished 5 grams of protein per kilo. The added protein was in the form of beef muscle (chuck). The figures refer to grams per 100 cc. serum. From the *Bulletin of the Johns Hopkins Hospital.*[11]

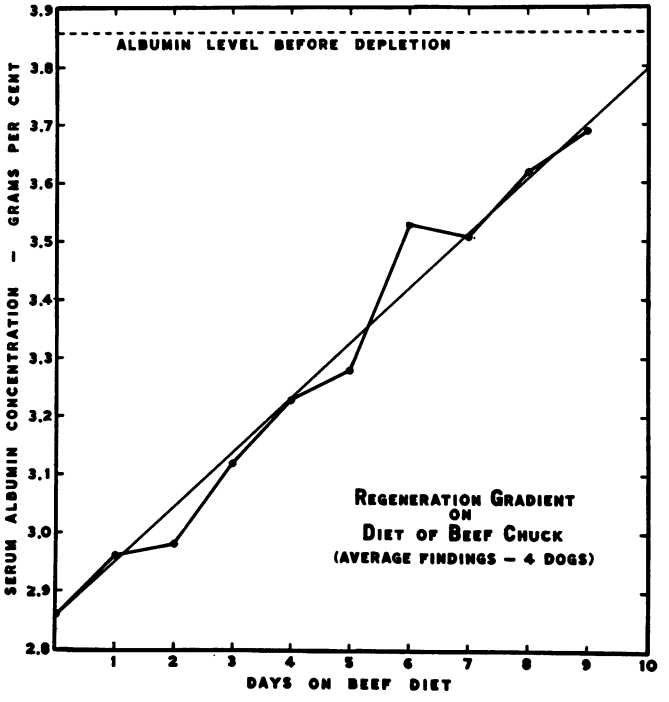

Chart 3—From the *Bulletin of the Johns Hopkins Hospital.*[11]

pletion exists. When the intake of food is constant, a fixed amount of the diet will be utilized each day for fabrication of albumin. The experiments are further informative because the result can be contrasted with the type of regeneration which occurs under other conditions, conditions in which we incline to the opinion that the body mechanism responsible for synthesizing albumin has been impaired. From this point of view the straight line regeneration curve can be regarded as exemplary of the type of regeneration to be expected when the mechanism for forming albumin is normal.

Within recent years the accumulation of clinical evidence has shown that chronic depletion of the serum albumin is apt to be associated with disease of the liver. In 1932 Thompson, Ziegler, and McQuarrie[12] reported a case of hypo-albuminemia and chronic edema in a girl, aged two and one-half years, who failed to exhibit a rise in serum albumin or alleviation from edema when given a diet upon which the nitrogen balance was positive. Since there was no loss of albumin in the urine to explain

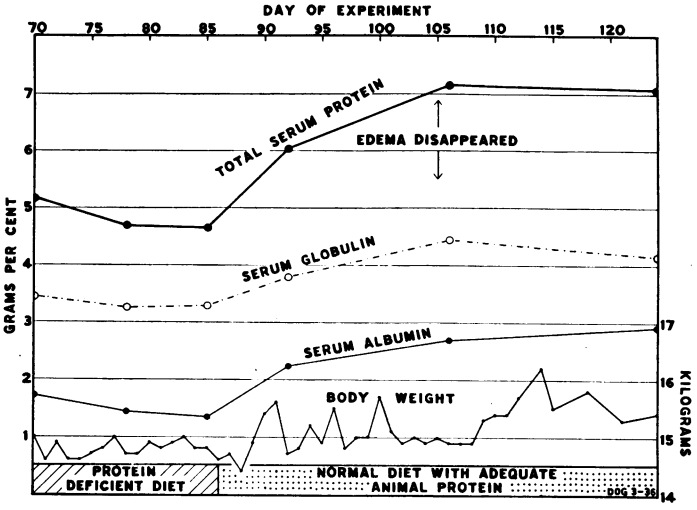

Chart 4—The regeneration of serum albumin in a dog debilitated by prolonged subsistence on the low protein diet. Before depletion the albumin concentration was 3.9 gm. per cent. The lowest level was 1.4 gm. per cent on the eighty-fifth day. After thirty-eight days of adequate feeding it had risen to 2.9 gm. per cent. From the *Journal of Experimental Medicine.*[6]

the serum deficit, the authors suspected a defect in the body in the process of manufacturing serum protein. In a later communication,[13] 1936, after the patient had come to autopsy, it was reported that she had suffered from primary atrophy of the liver. In 1933 a similar case was described by Myers and Taylor;[14] in this patient liver injury was suggested by the results of tests of liver function. In 1935 Myers and Keefer[15] discussed the relationship between depleted serum protein and cirrhosis of the liver; they explained the low serum protein as being the result of alteration in the function of the liver. The writer[16] has cited the case of an infant in whom low serum albumin and edema were apparently caused by syphilis which involved the liver extensively. Such circumstances make it relevant to inquire concerning the status of this function of the liver in other types of hypo-albuminemia, namely, in the types found in nephrosis and in chronic malnutrition.

That the mechanism for forming albumin may be impaired by prolonged malnutrition is suggested by several of our experiments with

dogs.[6, 11] When the time of maintenance on the low protein diet is lengthened to cover a period of several months, health and vitality are affected markedly, and replenishment of the albumin deficit is sometimes a protracted and difficult process. This state of affairs is illustrated by the experiment outlined in Chart 4. With this animal after eighty-five days on the low protein diet the albumin had fallen from 3.9 to 1.4 gm. per cent; the appetite had failed, vitality was low, and it seemed that life could not continue for more than a few days. From this time on an adequate and varied diet was offered daily; because of anorexia small amounts only were eaten at first and life was sustained by gavages of milk. A detailed record was not kept of the amount of food consumed daily but it is clear that the intake of both protein and energy increased slowly as strength and health returned. The behavior of the serum albumin concerns us here. During the first week the rise in concentration was rapid from 1.4 to 2.3 gm. per cent; during the next two weeks the rise to 2.7 gm. per cent was much slower; after three more weeks a level of only 2.9 gm. per cent had been attained. Thereafter, the gains continued but were extremely slow. After six months a level of 4.0 gm. per cent had been reached which was slightly above the previous concentration of health. With this animal, after an initial rapid response to food, subsequent albumin regeneration was slow and apparently independent of the quantity of food consumed. The form of the regeneration curve is impressively different from the straight line found in animals after short periods of depletion when health and vitality are not obviously impaired. The slow approach to normal suggests that replenishment is being delayed pending restoration to normal of the organ concerned in the synthesis of albumin.

If one accepts for the moment the possibility that liver injury was responsible for the delayed replenishment of albumin in this experiment, it is important to note that the injury was one which did not affect the early rate of synthesis but rather that it fixed at a subnormal value the level which could be reached. This observation may have considerable clinical significance. The constant low levels of albumin in patients with cirrhosis of the liver are similar; in such cases the initial response to an improvement in the diet is often meager, and complete recovery does not take place because the liver damage is permanent. In many cases of nutritional edema in humans, there is found upon institution of adequate feeding the same initially rapid and subsequently slow regeneration of

albumin as in our experiments with dogs. Such regeneration curves suggest that the synthesis of albumin may be impaired in man as in the dog by prolonged malnutrition. In the form of Bright's disease called "nephrosis," it has been customary to regard the extremely low levels of albumin in the serum as due entirely to loss of albumin in the urine. That defective synthesis of albumin may also play a part in maintaining the depletion is suggested by the constant nature of the albumin level in the serum in some patients over long periods when protein intake and degree of albuminuria are varying widely. In one of the cases reported by Keutmann and Bassett,[17] observations are recorded over a period of seven months during which the patient always received an adequate caloric intake. However, the diet was changed from time to time to include a number of food proteins at levels which varied from 68 to 180 grams per day. To a certain extent increases in dietary protein were associated with greater loss of protein in the urine; nevertheless, the higher intakes also produced significantly greater storage of nitrogen in the body. Under the circumstances it is noteworthy that during the entire seven months the concentration of protein in the serum remained almost constant at a level near 3.9 gm. per cent. Such constancy of serum protein concentration is difficult to understand in terms of defective kidney function alone. However, it is not difficult to explain if one admits the possibility of defective albumin synthesis of a type similar to that observed in our experiments with dogs.

In summary of Part I of this presentation: The administration of a low protein diet to a dog results in a progressive decrease in the concentration of albumin in the serum; the decline is at first rapid, later more gradual. A return to adequate feeding is followed by regeneration of the albumin. When the period of depletion has been short and the vitality of the animal is not impaired, the regeneration is rapid and on a constant diet follows a path of equal daily increments until the concentration of health has been regained. After long and debilitating periods of depletion, the regeneration, although initially rapid, is subsequently retarded and approaches the concentration of health very slowly. It has been suggested that the delayed type of regeneration may result from injury of the mechanism concerned in synthesizing albumin, a mechanism presumably located in the liver. Finally, data have been presented which intimate the existence of similar phenomena in several types of disease in humans.

Part II.

Your attention must now be turned to the importance in physiology of the albumin fraction of serum. I have already stated that it is not necessary to discuss fully the role of albumin in the control of fluid distribution at the capillary boundary since a number of aspects of this subject were covered in a previous Harvey Lecture. We may, however, record with pride that a New York physician, Epstein,[18] was the first to suggest that hypoproteinemia is the direct cause of edema in nephrosis and, we should fail to give honor where honor is due if we did not mention the great physiologist, Starling,[19] who first perceived the importance of the osmotic pressure of the protein of serum in preventing the passage of the fluids of plasma across the wall of the capillary into the tissues. It is interesting, however, that general acceptance of the postulates of Starling did not occur until Leiter,[20] 1928, and Barker and Kirk,[21] 1930, demonstrated that edema could be produced in otherwise healthy dogs by plasmapheresis, a mechanical method for removing plasma protein rapidly from the circulation.

That a correlation exists between the level of serum albumin and certain types of edema is now firmly established. With nephritic patients Moore and Van Slyke[22] found that an albumin concentration below 2.5 ± 0.2 gm. per cent was usually associated with edema. In malnutrition Bruckman and Peters[23] reported that edema almost always develops when the albumin falls below 3 per cent. In a study of nutritional edema in China,[24] dropsy was not observed when the albumin was greater than 3.0 per cent and was generally present when the level was less than 2.5 per cent. The relationship between albumin and edema in the experimental edema of dogs[25] is shown in Chart 5. The black dots represent analyses of plasma made when edema was present and the open circles refer to estimations before edema had developed or in a few instances after it had disappeared. Edema rarely appeared before the albumin was below 2 per cent; it was more often present than absent when the albumin was between 1 and 2 per cent; below 1 per cent edema was always present. Between globulin and edema no correlation can be discerned and the slight correlation in the total protein column obviously results from the albumin component.

The effectiveness of serum albumin in preventing edema depends upon its osmotic pressure. This pressure is exerted across the wall of the

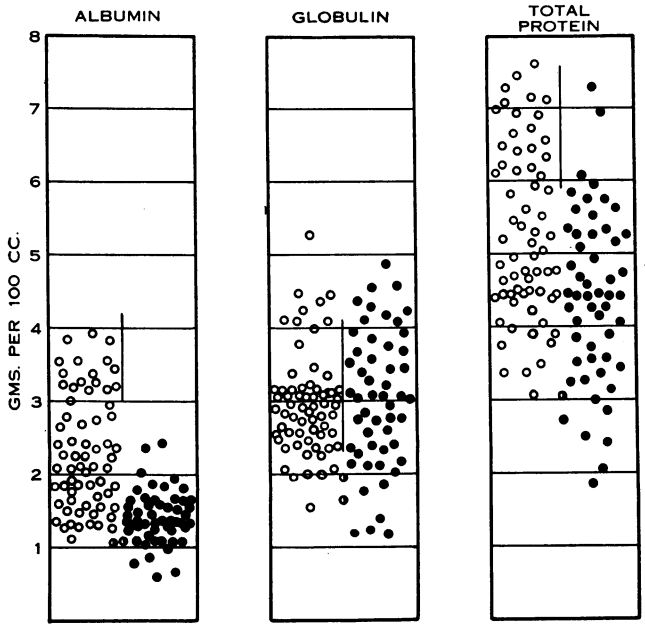

Chart 5—The relation between plasma protein concentration and edema in dogs. Open circles indicate estimations when no edema was present; black circles refer to determinations when edema was present; vertical lines in the middle of each column indicate the range of normal variation. From the *Journal of Clinical Investigation.*[5]

capillary because the capillary membrane is relatively impermeable to plasma protein while at the same time it is freely permeable to water, electrolytes, and other dissolved substances of low molecular weight. Under conditions where the capillary wall is damaged, the impermeability is destroyed and the edema which results represents simply the passage of plasma into the tissue spaces. Such forms of edema result from allergy and from inflammation. They need not concern us here. We must, however, be interested in the extent to which this protein traverses the wall of the capillary when there is no gross injury, that is, in defining as accurately as possible what we have called "relative impermeability." The available evidence is drawn from determinations of total protein in lymph[26,27] and in edema fluid. In view of Cecil Drinker's lecture[2] before this Society less than a year ago, I shall not discuss the subject of protein

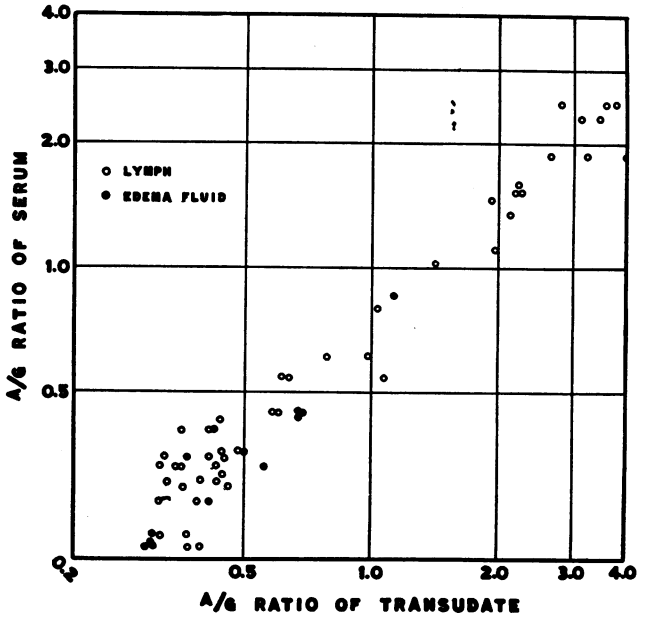

Chart 6—The relationship between the albumin: globulin ratios of concurrently collected samples of serum and transudate. A logarithmic scale has been used to avoid crowding of the data in the lower left-hand corner of chart.

in lymph. Much information concerning the protein in edema fluid has been assembled by Peters.[28] In nephrosis the fluid contains very little protein, usually less than 0.1 gm. per cent; in edema from myocardial failure the fluid contains more, generally from 0.5 to 2.0 gm. per cent. With nutritional edema in man, data assembled in our laboratory[16] indicate that the protein content of subcutaneous edema fluid varies from 0.1 to 0.6 gm. per cent. With experimental edema in dogs our experience is more extensive;[25] thirty samples from fourteen dogs have been examined. The range of protein concentration was from 0.02 to 0.72 gm. per cent; the average level was 0.23 gm. per cent and the median level was 0.17 gm. per cent.

The data just cited are of great value in tracing the behavior of total protein in plasma as it flows through the capillaries; they do not help specifically in following the course of the albumin fraction. It has been suggested that albumin because of its smaller molecular size may diffuse

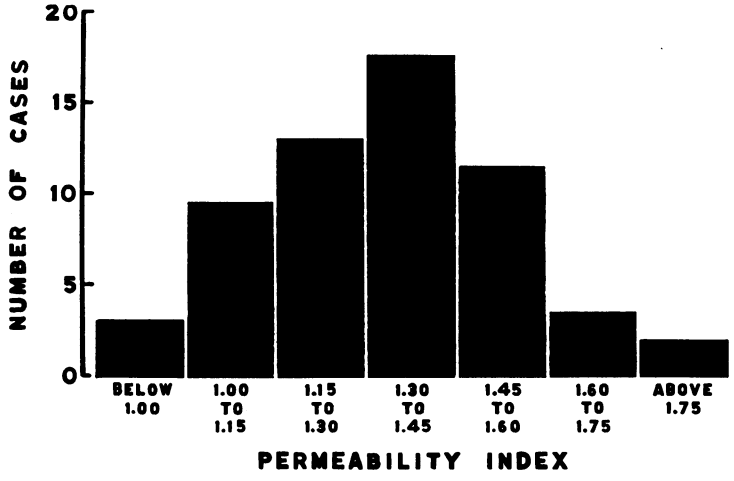

Chart 7

through the capillaries more freely than globulin and that the bulk of protein in edema fluid may be albumin. Because the osmotic pressure of albumin is much greater than that of globulin, it is desirable to have positive knowledge concerning the relative amounts of albumin and globulin in edema fluid. Information on this point has been supplied by Goettsch who in association with Kendall[29] developed a method for fractionating the serum proteins accurately in fluid samples of low protein content. The method is an outgrowth of fundamental work of Heidelberger and Kendall[30] and depends upon the precipitin reaction which appears when such fluids are brought into contact with specific rabbit antisera. Twelve samples of subcutaneous edema fluid and forty-eight samples of lymph from the extremities were obtained from normal and edematous dogs for this study; serum collected concurrently was also analyzed. Chart 6, prepared from these analyses, shows that the albumin: globulin ratio of a transudate is related closely to the albumin: globulin ratio of its corresponding serum. The concept is strongly supported that the albumin and globulin of lymph and edema fluid originate by filtration from the plasma. Incidentally it is shown that lymph and edema fluid are similar in this respect. In Chart 7 we have calculated for each transudate what may be called a "permeability index" and the indices have been arranged in the form of a frequency polygon. The index is secured when the A:G ratio of the transudate is divided by the A:G ratio of

the serum. When the permeability of the capillary is the same for albumin as it is for globulin, the index will have a value of one. As the index rises above unity a correspondingly greater differential permeability in favor of the smaller albumin molecule is indicated. The chart shows essentially a normal type of distribution for these permeability indices. The mode lies between 1.30 and 1.45; the average index is 1.35. The result tells us that albumin does traverse the capillary more readily than globulin but the difference is not extreme; if the ratio of albumin to globulin in serum is known the ratio in the transudate can be estimated by multiplying by 1.35. The chart shows three instances out of sixty in which the permeability index was less than one. I shall not attempt to explain these values other than to state that the figures were close to unity and that the slightly lower results may be merely an expression of analytical difficulties. They scarcely constitute grounds for believing that protein crosses the capillary wall by any other process than that of simple filtration.

Data of a similar nature for humans with edema are still meager. In seven cases where satisfactory analysis was possible the permeability indices ranged from 1.24 to 2.29 and averaged 1.55. There is little to suggest that in this respect the capillaries of the human do not behave like those of the dog. The reason why a larger number of analyses in the human is not available is part of another story which because of its importance can be touched on here. At the Babies Hospital most of the patients from whom adequate samples of edema fluid can be secured are children with nephrosis. It was while attempting to analyze samples from such patients that Dr. Goettsch[31] was led to discover that not only is the serum protein depleted in nephrosis but also that a part of the remaining protein is altered in a way which can be detected by the precipitin technic. This fact must become important in our ultimate understanding of the pathogenesis of nephrosis.

In addition to the data in Chart 7, three permeability indices have been determined for dog ascitic fluid. The values are 1.82, 1.98, and 2.18, all appreciably higher than the modal index for subcutaneous edema fluid and lymph. It will be recalled that fluid which leaves the blood to enter the subcutaneous tissue spaces must pass only one filtering layer of capillary endothelium; that which enters the abdominal cavity must in addition traverse the peritoneum. This fact may account for the higher indices recorded for ascitic fluid. If we suppose that capillary and peri-

toneum exert the same differential effect on filtration, a permeability index of 1.82 can be calculated for the two surfaces combined. The average of the three determined indices is 1.99.

I must now request a shift in the direction of your attention in order that we may consider another property of serum albumin, namely, that property which concerns its role in maintaining the volume of the blood. Not so many years ago the low concentration of protein in the serum of patients with nephrosis was interpreted to mean that both blood and tissues shared in the accumulation of edema; there was much talk of so-called "hydremic plethora." Probably Darrow,[32] 1926, was the first to record actual measurements which led to the conclusion that blood volume and plasma volume are below normal during the edematous stage of this disease. In view of contemporaneous work it is of interest that Darrow was able to reach his conclusion not on the basis of single measurements during the edematous phase but because volumes during edema and sometime after the disappearance of edema were compared. Other investigators[33] who studied the problem during this period, although able to dispose of the older concept of hydremic plethora, did not perceive the actual lowering in blood and plasma volumes. The findings of Darrow in nephrosis were later corroborated by Waterfield.[34] In 1932 Chang[35] in China in studying patients with nutritional edema found a close parallelism between blood volume and plasma protein. About the same time Lepore[36] made observations on dogs in the anemia colony of Whipple in Rochester; he found that the volume of the plasma varied consistently with changes in the concentration of albumin in the serum. Later, 1936, Melnick and Cowgill[37] reported that the parallelism between change in plasma volume and change in serum protein could be disturbed by significant alteration in the volume of red cells. You will see that we had been led to a similar conclusion.[38]

Chart 8 shows the average volume findings in ten of our dogs during maintenance on the low protein diet. Along with the decline in albumin which has already been described there is seen here to be a progressive fall in the total volume of the blood. The decline in red cell volume is likewise continuous but the plasma volume decreases for from twenty to thirty days only and thereafter remains at an approximately constant level. The failure of plasma volume to continue to fall does not mean that at this stage it is no longer sensitive to change in albumin concentration. In other experiments we have shown that at any degree of albumin

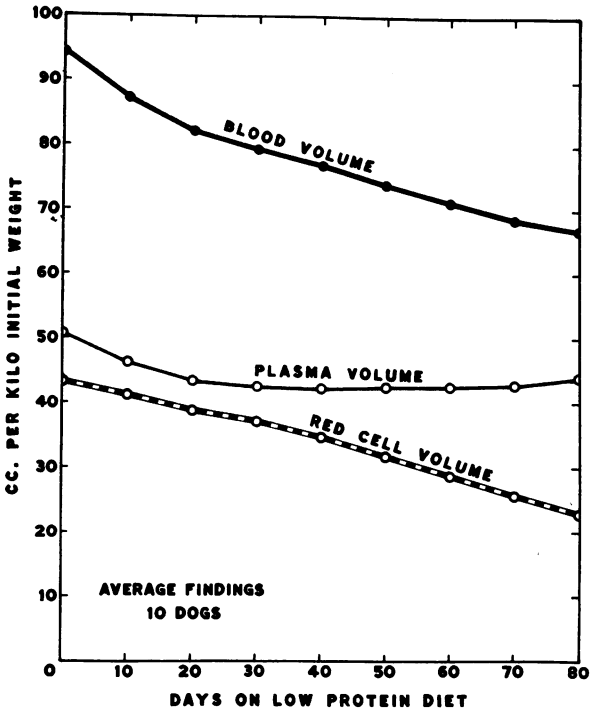

Chart 8—Changes in blood volume, plasma volume, and red cell volume during maintenance on the low protein diet. From the *Journal of Clinical Investigation.*[38]

depletion there will be an immediate rise in plasma volume if the albumin concentration is raised by transfusion either with serum or with solutions of serum albumin. We can imagine, however, that the continuous fall in blood volume which results from diminishing red cells has brought into play an opposing force strong enough to resist the effect of decreasing albumin concentration on plasma volume.

The fact that change in albumin concentration exerts an effect on the volume of the plasma assumes considerable importance when conditions are such that albumin is either rising or falling and it is desired to draw conclusions concerning some circulating substance from serial measurements of its concentration in blood. In Chart 8 it is seen that for some days the rate of decline is more rapid for plasma volume than

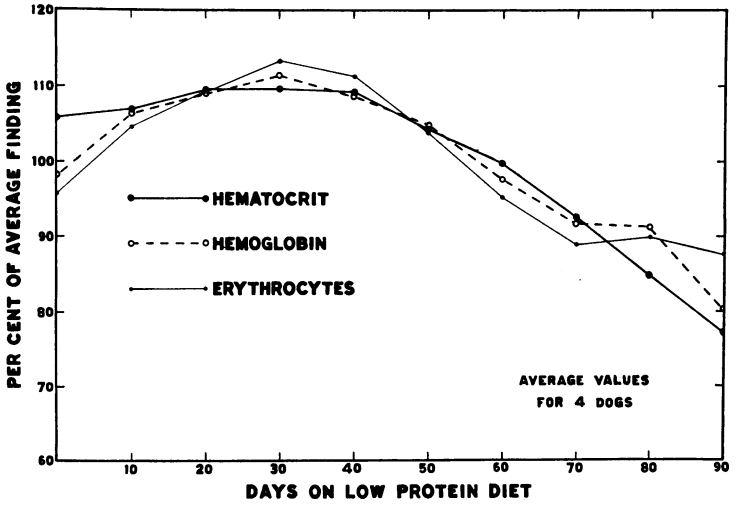

Chart 9—Variations in per cent cell volume, hemoglobin concentration, and number of erythrocytes during maintenance on the low protein diet. From the *Journal of Clinical Investigation.*[38]

for cell volume. During this period then the concentration of red cells must rise even though the total quantity is falling. This paradox is brought out in Chart 9 which presents the usual type of hemoglobin, erythrocyte, and hematocrit measurements in four dogs during maintenance on the deficient diet. If such measurements only were available one might be led to the curious and incorrect conclusion that ingestion of a deficient diet has a temporarily beneficial effect on hemoglobin and erythrocytes. I may add that examples of the fallacy of such reasoning are easily found in the clinical records of patients for even as illness and anorexia tend to go hand in hand so also does failure of appetite associate itself with a fall in serum albumin.

Part III.

The physician who investigates disease need feel no shame if his labor brings understanding even though there be no relief for his patient. Nevertheless, he must ultimately be impelled by at least a desire to strengthen the armaments of therapy. With this thought in mind I shall now turn to a consideration of measures which are being devised to re-

lieve the symptoms of albumin depletion. Of such symptoms the most conspicuous is dropsy. It is therefore fitting that we should consider first the attempts to remove edema by injecting into the circulation either albumin, as contained in serum or blood, or a substitute for albumin, like gum acacia. As a preliminary caution, however, we should be reminded that the phenomenon of diuresis is complicated and that it depends more directly upon renal activity than upon any particular distribution of fluid between circulation and tissues. A complete explanation of the initiation of diuresis is therefore not given when we say that this or that procedure has raised the colloid osmotic pressure of the blood. Indeed, we are forced to this conclusion by the observation that diuresis with elimination of edema occurs not infrequently when no evidence can be found that the osmotic pressure of the protein in the circulation has been altered. In such cases slight rises in albumin concentration in the plasma are often better interpreted as signs of blood concentration resulting from the diuresis than as the cause of its inception. In this connection I should like you to entertain a thought concerning the possible sequence of events when transfusions with serum, with blood, or with acacia are successful in initiating a diuresis. Experience indicates that these procedures, even when successful, usually produce slight and sometimes negligible effects on osmotic pressure. The evidence is strong, however, for an abrupt rise in plasma volume. It may well be that the rise in plasma volume is associated with an increase in renal blood flow and that the latter is responsible for stimulating kidney activity. In any case the possibility that some such sequence of events is involved will help in our understanding of observations that have been made in the clinic and in the laboratory.

Transfusions with blood to replenish the serum albumin have been attempted in many places. I know of no tabulation which permits appraisal of the efficacy of the procedure. Reports have appeared of cases in which the measure was successful in initiating diuresis but failure to obtain such action is a more common experience. Transfusions with serum alone have undoubtedly been used from time to time even as we at the Babies Hospital have occasionally turned to this procedure. Reports indicating conspicuous success have not appeared. Probably because the method involves the labor of handling large volumes of blood and because in many instances the blood must be purchased, the attention of investigators has been directed along other lines.

In 1932 Hartmann and his colleagues[39] in St. Louis suggested the use of acacia as a substitute for serum albumin in raising the osmotic pressure of plasma. By injecting this gum in large amounts intravenously Hartmann was successful in initiating diuresis in five out of six patients with nephrosis. Our own experience with this procedure has not been so rewarding; because severe constitutional reactions were encountered in the first few patients, we have not encouraged its use. Moreover, it has been reported[40] that much of the injected acacia is ultimately deposited in organs throughout the body, particularly in the liver, and Dick and his associates[41] have observed clinically that the continued use of acacia depresses still further the already depleted serum protein and is associated with an enlarged tender liver. Under such circumstances it is probably fair to assume that the procedure will not become popular even though its occasional use may be justified.

During the past summer a report has appeared of a more promising method of combatting serum albumin deficiency. Aldrich, Stokes, and other associates[42] have described a diuretic effect in nephrosis from injections of concentrated human blood serum. The serum used had been preserved in dried form by the lyophile process and was injected after redissolving so as to yield fourfold or fivefold concentration. In six of nine patients treatment was followed by complete and immediate diuresis. The authors did not feel, and indeed their analyses scarcely indicate, that the favorable results were due entirely to the osmotic action of the lyophile serum. They suggested that in addition some substance had been supplied which set off the patients' own mechanism of diuresis. Systemic reactions to the serum were usually mild but in two instances chills and high fever were seen. Dr. Lyttle has had some experience with this procedure at the Babies Hospital. Four children with nephrosis have been treated and in two of these the more severe type of reaction with chills and fever was noted. In only one case was satisfactory diuresis elicited. We were likewise led to conclude that the effect could not be ascribed to elevation of serum albumin concentration, but a sharp fall in the hematocrit reading following each injection suggested that the procedure did increase the plasma volume.

Because this form of treatment is destined to receive extensive trial in the near future it may be helpful to review one of a series of experiments performed several years ago[43] in which nutritional edema in the dog was treated by a similar method. Dog 2-07, Chart 10, had received

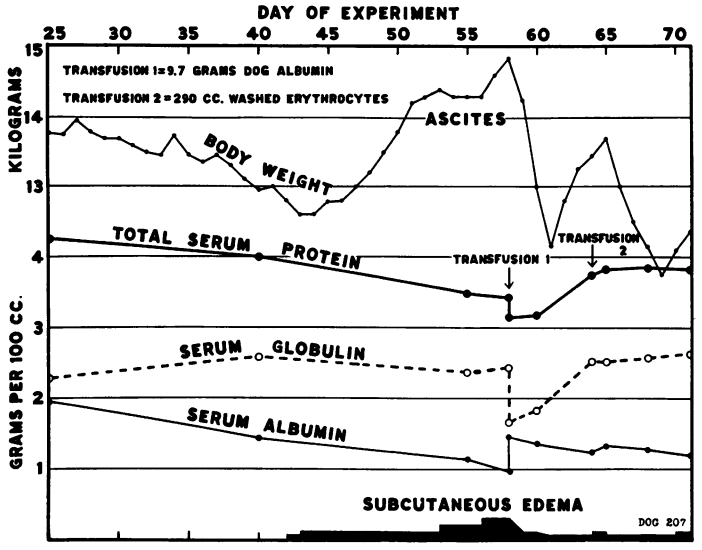

Chart 10—The effect of transfusion on serum proteins and edema in nutritional edema in a dog.

the low protein diet for fifty-eight days before treatment by transfusion was tried. The serum albumin had declined to less than 1 per cent and ascites had developed. At this point the animal was given intravenously 38 cc. of a 25.4 per cent solution of dog serum albumin. Before injection the plasma volume was measured and the total albumin in the circulation estimated to be 6.3 gm. The amount of albumin injected was 9.7 gm. The reaction was mild but similar in type to that observed in other dogs which have exhibited chills, diarrhea, and rapid irregular pulse of low volume. The subsequent clinical effect of diuresis which continued for three days was most encouraging. The changes in serum protein concentration are shown on the chart, a rise in albumin of only 0.5 gm. per cent and a fall in globulin great enough to produce an actual lowering in total protein. These changes were associated with a sharp fall in the hematocrit reading. Separate calculations based both upon the fall in hematocrit and upon the alteration in albumin indicate that the transfusion was followed by an abrupt increase in plasma volume from 630 to about 1,080 cc. We suspect that the reaction of shock may depend upon the sudden demand on cardiac reserve consequent upon such in-

creases in blood volume. The change in protein concentration in the serum is scarcely an adequate explanation for the degree of diuresis which ensued. Some light may be shed on this point by subsequent events. After the three-day diuresis the body weight again rose as edema reaccumulated. On the sixty-fifth day a second transfusion was given with 290 cc. of erythrocytes which had been washed in saline to remove the plasma. This time there was no untoward reaction and no demonstrable change in the serum proteins. Nevertheless, diuresis which lasted for four days was again instituted. The large volume of transfused red cells must have increased the total blood volume and this circumstance may have stimulated the kidney to diuresis.

If any conclusion is to be drawn from experiments of this type, it is that red cells and serum may both have some value in treatment. Transfusions with serum are invariably followed by a fall in relative cell volume and it would seem the part of wisdom to administer enough whole blood to prevent the fall. If it is true that untoward reactions may result from sudden increase in plasma volume, small and frequently repeated, rather than large, transfusions are indicated.

The futility of attempting to transfuse enough serum to replenish entirely the deficits in disease has been impressed on all who have tried the procedure. Albumin depletion is invariably a sign that the stores of nitrogen in many tissues are likewise depleted and it may be impossible to prevent an exchange of protein between circulation and other tissues when large volumes of serum are injected. Whipple and his associates[44] have already indicated the possibility of such an exchange by demonstrating in the dog that the protein requirement of the entire body can be met by massive transfusions of plasma. The only practical way in the clinic of meeting the full demand of the body for protein is through the diet.

From time to time and in different places the idea has evolved that some dietary proteins may be more efficient than others in supplying the needs of the plasma. There is opportunity merely to mention the convincing and provocative work of Whipple[45] who with his associates has utilized the technic of plasmapheresis to show that qualitative differences exist among different foods with respect to their utilization in forming serum protein. The remaining portion of this lecture must be devoted to experiments having a similar purpose now under way in our own laboratory.[11, 46] These experiments have as their ultimate goal the

classification of the more important food proteins with respect to their ability to promote the formation of serum albumin.

The method which is being used to assay the food proteins is simple. A healthy dog is selected and placed under daily observation for six days on a standard diet of adequate protein content. The standard diet is a mixture of 900 grams of our basal low protein ration with 90 grams of casein. The concentration of albumin in the serum is then measured; the casein is removed from the diet and the period of depletion of the serum albumin is begun. After three weeks the serum albumin is again determined and the period of regeneration started on a diet composed of a mixture of the basal diet and the food protein to be tested. The mixture is fed at an energy level of eighty calories per kilo and at a protein level of 2.5 grams per kilo. After one week on this diet the final measurement of serum albumin concentration is made.

The initial albumin analysis is a control determination only; the result has no direct part in evaluating the assay. The difference between the third and the second analyses gives the rise in serum albumin concentration during the week when the test protein was fed. In twenty-three control experiments in which the basal low protein diet was actually fed longer than three weeks, the average decline in albumin during the fourth week was 0.15 gm. per cent. Since the test food should receive credit for preventing this decline, the "assay value" is calculated as the rise in albumin concentration plus 0.15. When assay values are determined for the same food on a number of different animals, the range of biological variation among the results will be fairly wide. It follows that a series of assays must be made before an average value, called "the potency value," can safely be assigned to any one protein.

Table III shows the results of twelve assays of casein by this method. The columns in the table show the albumin levels at the start of each experiment, the levels reached at the end of three weeks of low protein feeding, and the final levels attained after one week when casein was again incorporated in the diet. In the next two columns the loss in concentration during the period of depletion is compared with the subsequent gain. The assay values are obtained by adding the maintenance allowance, 0.15, to the figures which represent gains in grams per cent. The potency value or average of the assay values is 0.388. Although the majority of the assay values are in fairly good agreement with the potency value, I must direct your attention to the extreme variability in

TABLE III

ASSAYS OF *CASEIN* FOR ALBUMIN FORMATION

Dog	*Serum Albumin per 100 cc.*					*ASSAY VALUE*
	Initial	*Depletion*	*Regeneration*	*Loss*	*Gain*	
	gram	*gram*	*gram*	*gram*	*gram*	
B-12	3.81	2.63	2.55	1.18	−.08	0.07
5-7	3.61	2.76	2.79	0.85	0.03	0.18
5-31	4.07	3.04	3.25	1.03	0.21	0.36
7-27	3.40	2.27	2.51	1.13	0.24	0.39
6-35	2.89	1.90	2.14	0.99	0.24	0.39
8-04	3.38	2.52	2.77	0.86	0.25	0.40
9-51	3.46	2.73	2.99	0.73	0.26	0.41
7-28	3.16	2.40	2.70	0.76	0.30	0.45
7-47	3.44	2.47	2.78	0.97	0.31	0.46
3-0	3.80	2.91	3.23	0.89	0.32	0.47
9-49	4.60	3.20	3.54	1.40	0.34	0.49
3-36	3.42	2.66	3.09	0.76	0.43	0.58

POTENCY VALUE: 0.388 ± 0.027 (P.E.)

From the *Bulletin of the Johns Hopkins Hospital.*[11]

TABLE IV

ASSAYS OF *BEEF SERUM* FOR ALBUMIN FORMATION

Dog	*Serum Albumin per 100 cc.*					*ASSAY VALUE*
	Initial	*Depletion*	*Regeneration*	*Loss*	*Gain*	
	gram	*gram*	*gram*	*gram*	*gram*	
1-68*	3.39	2.61	2.80	0.78	0.19	0.42
5-31	4.14	3.23	3.66	0.91	0.43	0.58
3-0	3.69	2.83	3.28	0.86	0.45	0.60
7-31	3.58	2.53	3.02	1.05	0.49	0.64
9-51	3.47	2.59	3.10	0.88	0.51	0.66
2-05*	3.63	2.29	2.71	1.34	0.42	0.74
6-35	3.26	2.42	3.11	0.84	0.69	0.84
9-52	2.61	1.45	2.20	1.16	0.75	0.90
3-36	3.72	2.74	3.63	0.98	0.89	1.04
1-69*	3.02	2.19	2.88	0.83	0.69	1.12
1-55	3.61	2.39	3.51	1.22	1.12	1.27

POTENCY VALUE: 0.801 ± 0.053 (P.E.)

* In these experiments the regeneration period lasted for five instead of seven days. Calculation of the assay values is explained in the original publications. From the *Bulletin of the Johns Hopkins Hospital.*[16]

a few experiments; the extreme cases give a total range among the assay values from 0.07 to 0.58. This tendency for a few animals to diverge widely from average behavior constitutes the major difficulty encountered in experiments of this type. So far we have not learned how to overcome the difficulty except by performing a fairly large number of assays with each food.

Table IV shows the results with the most potent food protein we

TABLE V

	Gelatin	*Casein*	*Beef Liver*	*Beef Chuck*	*Egg White*
Beef Serum (11) 0.801	0.0001	0.0001	0.0003	0.0007	0.042
Egg White (11) 0.613	0.0001	0.0003	0.0026	0.0090	
Beef Chuck (12) 0.475	0.0001	0.098	0.42		
Beef Liver (12) 0.436	0.0001	0.38			
Casein (12) 0.388	0.0001				
Gelatin (4) –.093					

have tested so far, namely, beef serum. Eleven assays have been performed. Only the two lowest assay values overlap with the casein series. The remaining values are higher than any recorded with casein. The potency value for beef serum, 0.801, is more than twice that for casein.

At the present time assays of six foods have been completed. The values for the six foods arranged in order of potency are given in the left-hand column of Table V. Beef serum is first, with a potency value of 0.801. It is significant that Whipple came to the same conclusion by a wholly different method of assay. There follow: egg white (11 assays), 0.613; beef muscle (12 assays), 0.475; beef liver (12 assays), 0.436; casein (12 assays), 0.388; gelatin (4 assays),—0.093. In three of the four assays of gelatin, the albumin level at the end of a week of feeding this protein was less than would have been expected if no protein at all had been fed.

Gelatin therefore appears in the table with a negative value. The remaining columns of the table are given over to a statistical tabulation of the probability that the difference in potency value between any two of the foods is significant. When the tabulated value of the probability integral is less than 0.05, a significant difference may be regarded as established. Beef serum is clearly more potent than casein, beef liver, and beef muscle; the difference between beef serum and egg white is barely significant. Egg white, however, is secure in second place. There is no question concerning the absolute inferiority of gelatin. However, a real difference between beef muscle, beef liver, and casein has not been shown.

The clinical usefulness of assays of this kind remains to be demonstrated. Because physicians have sometimes failed to observe startling distinctions among different food proteins in humans with nephrosis does not mean that qualitative differences such as we have shown to hold for the dog do not also apply in man. The suggestion has already been made that in disease the replenishment of albumin may sometimes be delayed by injury of the organ concerned in synthesis. Such injury when present will complicate greatly the interpretation of dietary experiments on the ward.

* * * * * * * * * * *

In summary: I have traced for you some of the features which characterize the behavior of the albumin fraction of serum, the manner in which it becomes depleted when dietary protein is inadequate and the path of regeneration when adequate feeding is resumed. I have discussed the part in physiology served by albumin by virtue of the osmotic pressure which it exerts, namely, its function in protecting the circulation as blood flows through the capillaries so as to prevent edema and its role in maintaining the volume of the blood. And finally, I have described experiments which look toward the more intelligent management of patients who suffer from depletion of serum albumin.

In conclusion: The material from which this Harvey Lecture was composed was chosen to trace the course of research in a single experimental laboratory. You have honored me in asking that I tell the results of the work, and in turn it becomes my privilege to divide the honor with those who have gone with me: with Dr. Elvira Goettsch who has been my associate for the past seven years; with several technicians who have endured the tedium of thousands of Kjeldahl analyses;

with Boris Gagarin who with devotion has cared for my dogs; and finally with all of those friends and associates at the Medical Center who by conversation and suggestion have been responsible for crystallizing the ideas which have been expressed.

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